GCE

Rewarding Learning

Revised GCE

Biology

A2 3: Practical Guidance Document

For first teaching from September 2016

Unit A2 3: Practical Skills in Biology

The information provided here is intended to provide support for teachers delivering the revised CCEA GCE Biology course (first teaching from September 2016).

For most practical tasks this guidance provides detailed background information rather than providing a series of 'recipe' practical investigations (although these are included for some tasks). The internet (for example www.nuffieldfoundation.org) and many other sources provide additional information, in carrying out 'A' level Biology tasks.

Note: This document does not provide health and safety information for the safe carrying out of practical tasks identified in the specification. It is the responsibility of teachers to ensure that they and their students are aware of any health and safety issues that are relevant in any particular task.

Investigate microorganisms involving aseptic techniques

Note: as with all GCE practical activities it is essential that students are fully briefed on all relevant safety procedures in advance of working with cultured microorganisms.

Aseptic technique – this describes the range of techniques used when culturing microorganisms to prevent contamination. This includes preventing contamination of the culture and contamination of the individuals involved.

Solid agar or liquid broth can be used to culture microorganisms and care is needed when transferring microorganisms to Petri dishes or other containers for investigation.

Procedure for transferring microorganisms:

- Metal inoculating or disposable plastic loops can be used

- If using a metal inoculating loop it is necessary to 'flame' the loop in the hottest part of a Bunsen flame until it becomes red-hot. After sterilising the metal loop it is necessary to air cool the loop as when red-hot it would kill any microorganisms it comes into contact with. After transfer of microorganisms it is necessary to re-sterilise the loop. However, for safety reasons it is important not to create a microorganism-rich aerosol when doing this. Once used, disposable plastic loops should be discarded into a solution of disinfectant.

- When transferring microorganisms from a culture bottle to a Petri dish (or a fresh culture bottle) the lid of the bottle should be held in the same hand that holds the culture bottle and not allowed to touch the bench, with the other hand holding the inoculating loop. Immediately after opening the culture bottle it should be quickly passed through the Bunsen flame to sterilise the lid region; this should be repeated immediately before putting the lid of the culture bottle back on.

- When transferring the microorganisms to a Petri dish, the lid of the dish should be only raised enough to allow the microorganisms to be added to the agar.

Alternatively, a spread plate method can be used, when cells in suspension are used in inoculation. In

this case an L-shaped spreader is used to spread the inoculating bacteria over the surface of the agar. Plastic disposable spreaders are a suitable alternative to the glass spreaders that require sterilisation before and after use.

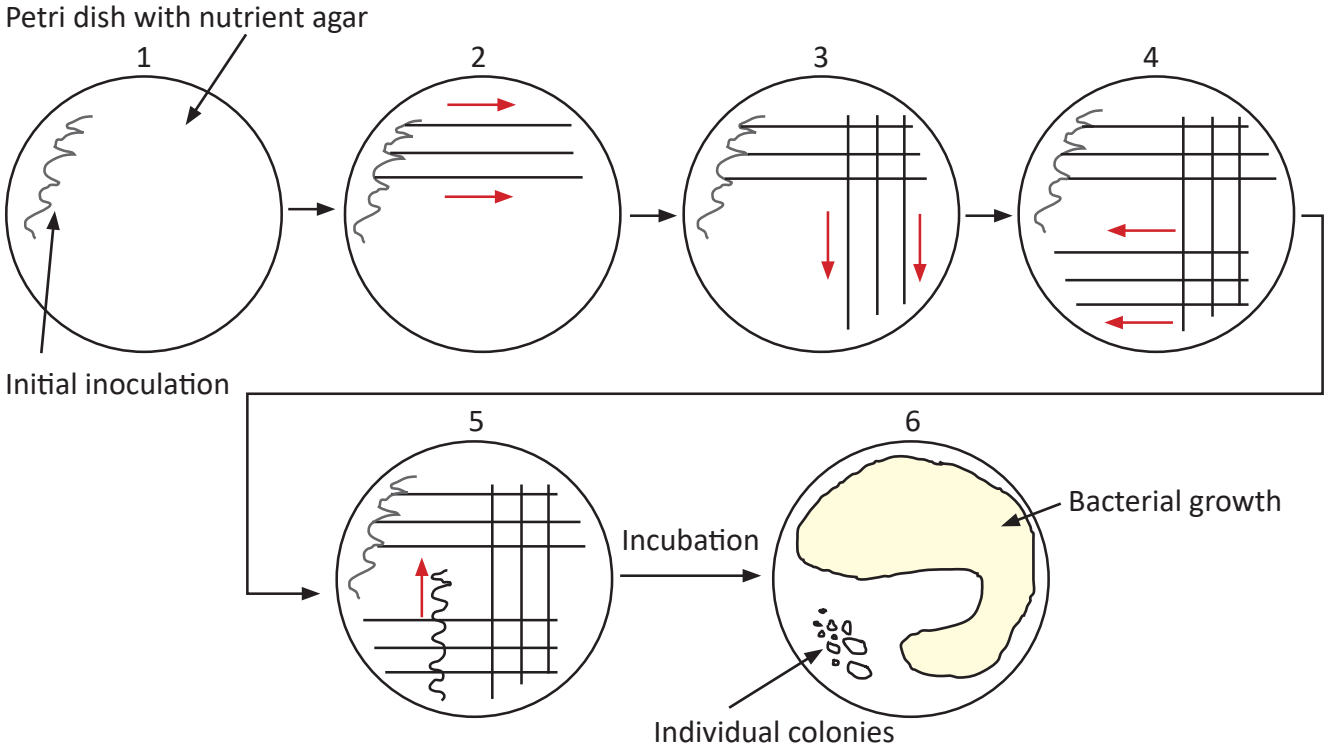
Petri dishes should be labelled on the outside of the base (and not the lid). They should be incubated upside down in an incubator at an appropriate temperature for the microorganism concerned.

1. Preparation of a streak plate to isolate single colonies

This technique is commonly used to isolate single colonies of microbial cells, i.e. a pure culture of cells all of which share the same parental cell.

Procedure:

- Using an inoculating loop spread the microorganisms over a small section of the agar in the Petri dish.
- Then with a new / sterilised inoculating loop 'streak' several lines of microorganism across the agar at an angle, taking care not to allow the separate 'lines' to overlap.
- Repeat once or twice making sure that a sterile loop is used on each occasion.
- Make a final single streak as shown in the diagram below.
- Incubate the Petri dish at a suitable temperature. After 24 hours or so it should be possible to identify isolated pure colonies.



Preparing a streak plate

Evidence: a brief outline of method and a drawing or photograph of the Petri dish following incubation with a brief discussion.

2. Investigate the antimicrobial properties of plants

Many plants have a range of defences against microorganisms. These help prevent decay and further loss in parts of plants, for example leaves, which become damaged in their natural environment.

Many common woodland plants such as wood anemone, bluebell, lesser celandine and, in particular, wild garlic produce compounds that prevent or reduce fungal and/or bacterial growth.

Procedure:

- Grind up leaves of, for example wild garlic, lesser celandine and wood anemone with a small amount of water in separate mortars.

- Prepare some agar plates (Petri dishes) and spread a fungus or bacterium culture on the plate using appropriate aseptic techniques. The fungus *Pythium debaryanum* or Bacillus bacteria are suitable for this purpose. Soak a small disk of filter paper with each plant extract, dry and place on the agar in the Petri dish.

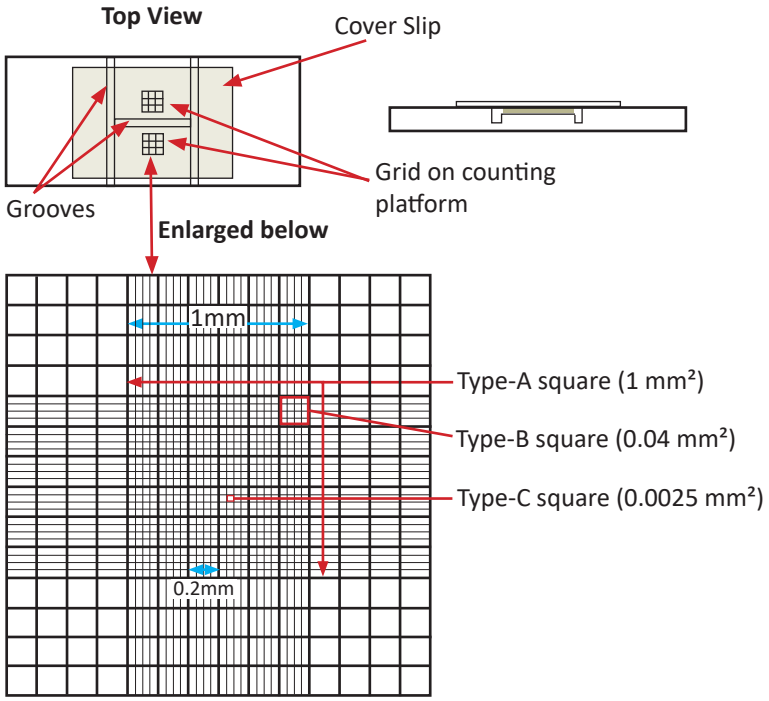
- Incubate for 24 – 48 hours and compare results in the different Petri dishes.

Alternatively, instead of comparing antimicrobial properties in different plants it is possible to compare the effect of extract concentration, temperature of incubation and many other variables.

Evidence: a drawing or photograph of the Petri dish following incubation and a brief conclusion.

3. Investigating microbial, for example yeast, population growth using a haemocytometer

The haemocytometer is a modified slide that is used for counting cell numbers. Essentially there are three different sizes of square that can be used when counting as shown in the diagram below.



The haemocytometer

As the distance between the base of the depression that contains the cell suspension and the cover slip is 0.1 mm then the volume of cell suspension in each type of square is:

- Type-A = 1 mm^2 x 0.1 mm = 0.1 mm^3
- Type-B = 0.04 mm^2 x 0.1 mm = 0.004 mm^3
- Type-C = 0.0025 mm^2 x 0.1 mm = 0.00025 mm^3

Once the average number of cells in a particular volume (square) has been calculated it is possible to then calculate the number per mm^3 or in any volume.

When counting the cells in a particular square it is important to use a consistent approach when counting cells that lie across a square boundary. For example, counting cells on the top and left grid lines but not counting those on the bottom and right grid lines (the north-west rule).

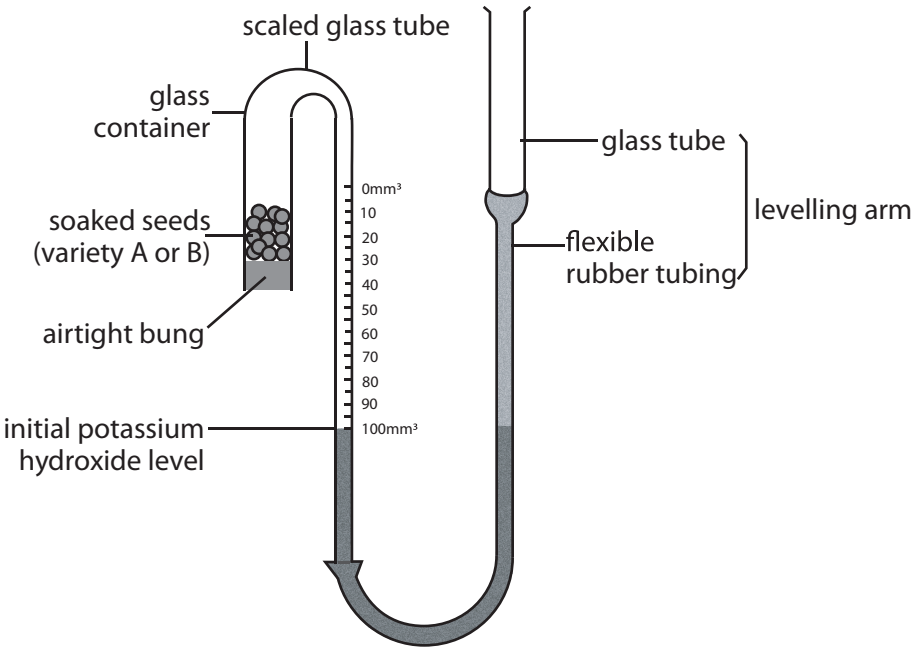
When investigating population growth in yeast in the laboratory there are many hypotheses that can be tested. For example, the effect of availability of oxygen (for example conical flask size), the effect of temperature or food substrate.

Evidence: table showing results from haemocytometer readings and calculations of cell number per unit volume of suspensions used, followed by a brief explanation / conclusion.

4. Use of a respirometer to calculate oxygen uptake, carbon dioxide production and / or RQ values

(Note: students can use data generated from a respirometer set up by the teacher / lecturer. However, students are expected to complete their own calculations on the data gained).

Oxygen uptake in living organisms is calculated by adding potassium hydroxide (KOH) to the respirometer. When the respirometer is 'closed' the living organisms, for example germinating peas, maggots, respire taking in oxygen from within the system. The carbon dioxide produced is absorbed by the potassium hydroxide. The consequent reduction in pressure causes the liquid / dye in the respirometer to move in the direction of the biological material. The distance (volume) moved represents the oxygen used in respiration.



Respirometer

Carbon dioxide (CO_2)production can be calculated by repeating the process but replacing the KOH with water. The respirometer needs to be left for the same length of time and in the same conditions as that for measuring oxygen (O_2)uptake. This time the CO_2 produced is not absorbed. If there is no change in the movement of the liquid in the manometer or calibrated scale then the volume of CO_2 produced is the same as the volume of O_2 absorbed. If the liquid moves closer to the biological material then there is more O_2 absorbed than CO_2 produced and if it moves further away there is more CO_2 produced than O_2 absorbed. The actual amount of CO_2 produced can be calculated taking account of this and the results recorded when calculating O_2 uptake.

RQ values can be calculated by dividing the value for CO_2 production by the value for O_2 uptake.

Evidence: table showing results from respirometer readings and calculations of O_2 uptake and / or CO_2 production and / or RQ values, followed by a brief explanation / conclusion.

5. Demonstrating the role of hydrogen acceptors using redox indicator (in photosynthesis or respiration)

It is possible to use redox indicators to demonstrate dehydrogenation in respiration as redox indicators such as DCPIP or methylene blue change colour as they become reduced.

Redox indicators can be used to demonstrate photolysis using isolated chloroplasts. After grinding fresh (lettuce) leaves in ice-cold buffer, chloroplasts in the homogenate can be separated using centrifugation. The isolated chloroplasts can be treated as outlined in the table below.

Tube	Treatment	Colour change
A	water + DCPIP in bright light (control)	no change
B	chloroplast suspension + DCPIP in bright light	blue/green to green
C	chloroplast suspension + DCPIP in darkness	no change

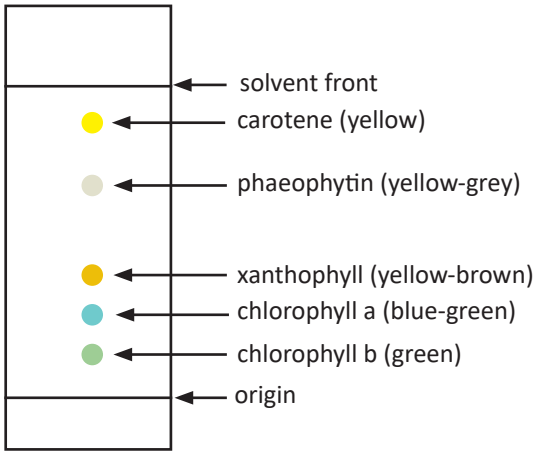
Evidence: table of results, followed by a brief explanation / conclusion.

6. Use paper chromatography to identify plant pigments

The basic principles of chromatography, how to prepare the chromatogram and how to calculate Rf values are covered in the chromatography topic in the AS section of practical support.

The procedure involves grinding some plant leaves, for example nettle, in a mortar with a small amount of solvent/acetone. If necessary a very small amount of sand can be added to aid the grinding process. The plant material should be macerated to the extent that there is enough of a concentrated dark green liquid to enable the chromatogram to be 'spotted'. Filter the extract to remove larger pieces of plant debris.

Following the development of a concentrated 'spot', the chromatogram should be left to run until the solvent front is close to the top. At this stage remove the chromatogram from the solvent and mark the solvent front. Allow to dry and a number of pigments will become visible.



Chromatogram of the plant pigments from a typical plant

A table similar to the table below can be used to record results.

Pigment	Distance travelled by solvent front/ mm	Distance travelled by pigment/mm	Rf value of pigment	Colour of pigment	Name of pigment
A					
B					
C					
D					
E					

The colours and Rf values of the common pigments found in most plants will be similar to the values in the table below. (Exact Rf values depends on a number of factors including the solvent used.)

Name of Pigment	Colour	Rf value
Carotene	Yellow	0.95
Phaeophytin	Yellow-grey	0.83
Xanthophyll	Yellow-brown	0.71
Chlorophyll a	Blue-green	0.65
Chlorophyll b	Green	0.45

Evidence: Table of results, copy (photograph) of chromatogram, and calculations of Rf values to identify particular plant pigments.

7. Working with DNA

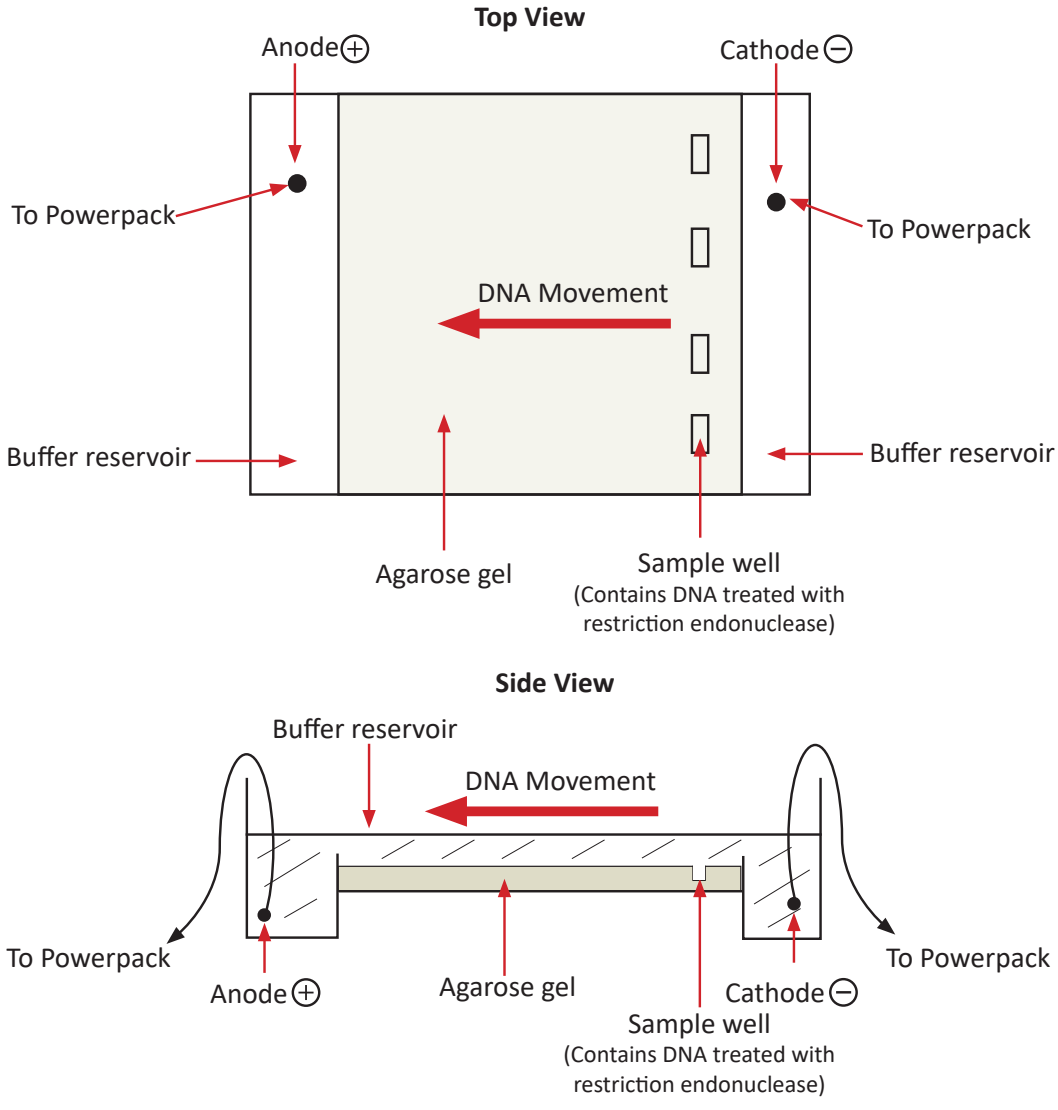
(a) Carry out gel electrophoresis of DNA

Electrophoresis is a process that separates sections of DNA as a consequence of their differential movement in an electric field. Essentially current flows in a buffer solution through an agarose gel between the cathode and an anode in the electrophoresis set-up.

Sections of DNA travel at different speeds (and different distances) through the agarose, depending on their size (and electric charge). The agarose contains small pores, so that the gel effectively 'sieves' the sections of DNA as they travel through it.

At one end of the electrophoresis system 'wells' are sunk into the agarose gel. Typically these are developed by placing a 'comb' in situ and allowing the agarose to set around it. When the 'comb' is removed after the agarose has set the wells remain. The DNA sections to undergo electrophoresis are placed in the wells. Typically different wells contain DNA that has been treated with different restriction enzymes.

After a certain period of time dye is added to the system to allow the DNA to be seen.



Evidence: a description of results obtained (or photograph) followed by a brief conclusion / analysis of results obtained.

(b) **Extraction of DNA –** DNA can be extracted from a range of plant or animal material in a number of ways. For example, it can be extracted from plant tissue by carrying out the following procedure.

Procedure:

- Add a pinch of salt and a small amount of cold water to the plant material, for example spinach.
- Use a blender to break up the plant material; alternatively grind in a mortar and pestle.
- Strain off the larger pieces of plant material and mix the resulting macerate with detergent (or liquid soap) to break down the cell membranes. Leave for 5 -10 minutes.
- Add protease enzyme to separate the DNA from the chromosomes (pineapple juice or meat tenderiser does the same job).
- Gently add a layer of very cold ethanol. This causes the DNA to precipitate out of the solution. The DNA can be seen as a series of white strands.

Note: There are many different methods of extracting DNA from plant and animal tissue and many can be found on the Internet.

Evidence: a brief description of method used and an outline of the outcome with possible photograph.

8. Dissect a small animal, animal organ or part of a plant

As indicated in the specification the emphasis is on developing manipulative skills so the practical task must be reasonably complex. Rat dissections, insect mouthparts and similar dissections are appropriate. A dissection of the heart can only be included if it was not used as evidence of a required practical task at AS by the candidate.

A straightforward dissection that does not involve ordering additional biological material is to do a leaf scrape to produce an epidermal layer for examination under the microscope.

Leaf scrape procedure:

- Place a grass leaf flat on a microscope slide, top surface up, and irrigate with water (no cover slip at this stage). Use a species with flat rather than rounded leaves.
- Hold the end of the leaf with the thumb of one hand and using a safety razor held vertically in the other hand gentle but not too slowly slide the razor down the grass blade. This will remove a number of cell layers from the top of the leaf.
- Add water as necessary to reduce friction between the vertical blade and the leaf.
- Repeat a number of times – it is important that the razor strokes only occur in the one direction along the leaf (and away from the thumb holding the leaf in place).
- After a number of strokes the leaf will appear much lighter in appearance as fewer cell layers are left.
- When the leaf is no longer green and has a more transparent appearance the epidermal layer and cuticle are all that remain.
- Examine under the microscope. Depending on species, it should be possible to observe stomata, epidermal cells and leaf hairs.

Evidence: Labelled drawing of leaf scrape (or as appropriate for dissection, for example respiratory or digestive system in a rat).

COUNCIL FOR THE **CURRICULUM, EXAMINATIONS** AND **ASSESSMENT**
29 Clarendon Road, Clarendon Dock, Belfast BT1 3BG
Tel: +44 (0)28 9026 1200 Fax: +44 (0)28 9026 1234
Email: info@ccea.org.uk Web: www.ccea.org.uk

Rewarding Learning